# Studies Concerning Nutritional Muscular Dystrophy in Guinea Pigs and Rabbits, and the Role of Vitamin C in Reproduction

DISSERTATION

Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Pure Science of Columbia University

BY

MARIANNE GOETTSCH, B.A., M.A.

NEW YORK CITY

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# RELATIONSHIP BETWEEN VITAMIN C AND SOME PHASES OF REPRODUCTION IN THE GUINEA PIG[1,2]

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Relatively few studies have been made upon the dietary needs of the guinea pig for reproduction. Papanicoloau and Stockard (1919–1920) discovered that inanition prolonged the dioestrous interval of the ovulation cycle. Ingier (1915) in a series of experiments in which guinea pigs were deprived of vitamin C during different stages of gestation, established that the gravid animal was more susceptible to scurvy than others, that pronounced scurvy might be produced in the fetus as early as the tenth to the fifteenth day after the diet of the mother had been changed, and that frequently the fetus was born prematurely or still-born. These facts were confirmed by Reyher, Walkoff, and Walkoff (1928). Linsay and Medes (1924–1925), (1926) reported that male guinea pigs with mild chronic scurvy did not reproduce and they described extensive histological changes in the germinal epithelium of the testes.

In the course of some experiments conducted at the Pharmacological Laboratory of the Pharmaceutical Society of Great Britain, on the assay of vitamin C, two observations were made concerning the reproduction of guinea pigs, which led to the present investigation. First, females on the Zilva scorbutic diet with various daily doses of orange juice, including doses high enough to prevent scorbutic lesions in the teeth as described by Höjer (1924), (1926), failed to manifest oestrus during 42 days of experiment, and secondly, smears from the epididymis of males, dying of scurvy, contained motile sperm. That the oestrous rhythm is a sensitive indicator of the nutritional state of an animal was pointed out by Papanicoloau and Stockard (1919–1920) in some inanition experiments with the guinea pig, and by Evans and Bishop (1922) in a study of nutritional deficiencies in the rat. When, in the early experiments with the Zilva scorbutic diet, even those females receiving a protective dose (3 cc.) of orange juice

[1] The data in this paper are taken from a dissertation submitted by the author in partial fulfilment of the requirement for the degree of Doctor of Philosophy, Columbia University, 1930.

[2] This work was aided by the Research Grant from the Chemical Foundation to the Department of Biological Chemistry.

failed to exhibit oestrus, it was clearly apparent that the diet must be lacking in some necessary constituent, other than vitamin C. For the present experiments, therefore, a modification of the Sherman (1922) scorbutic diet was chosen.

EXPERIMENTAL. The following diet 2 was used:

| | Parts |
|---|---|
| Equal volumes Pillsbury's bran and quaker oats | 580 |
| Merrell Soule skimmed milk powder baked 2 hrs. at 110°C. | 300 |
| Milk fat from sweet butter | 100 |
| NaCl | 10 |
| Cod liver oil (Mead's) | 10 |

(The diet was freshly prepared 2 or 3 times a week)

Supplementary (see table 1) doses of fresh orange juice were administered daily by pipette.

TABLE 1

*Length in days of successive oestrous cycles of guinea pigs on the scorbutic diet with decreasing amounts of vitamin C*

| NUMBER OF GUINEA PIG | STOCK DIET | SCORBUTIC DIET NO. 2 | | | | REMARKS |
|---|---|---|---|---|---|---|
| | | 3 cc. orange juice | ½ cc. orange juice | ¼ cc. orange juice | ⅛ cc. orange juice | |
| ♀ 9 | 17, 18, 16 | 31, 16, 15 | 16, 16, 15 | 16, 16, 16 | Decline | Cured; 3 cc. orange juice |
| ♀ 10 | 18, 17, 15<br>19, 16, 14 | 14, 15, 18 | 18, 14<br>19, 14 | 15, 16<br>Decline | | Severe scurvy at autopsy |
| ♀ 26 | 20, 15, 17 | 15, 15, 15 | 15, 15, 15 | 15, 15, 15 | 15<br>Decline | Severe scurvy at autopsy |

Guinea pigs weighing 250 to 300 grams were secured from a dealer and were bedded on wood shavings. Their daily weight as well as food consumption was recorded. The females were examined daily for the vaginal membrane of dioestrus, described by Stockard and Papanicoloau (1919) and the occurrence of oestrus was confirmed by a microscopic study of the smears obtained when the membrane was found to be ruptured (1918).

*Effect of vitamin C deprivation upon oestrus rhythm.* Young control guinea pigs kept on the stock diet of oats, bran, hay, and greens, had more irregular cycles than those described by Stockard and Papanicoloau (1918), who reported a mean length of 15.74 days with variations no greater than from 15 to 17 days. The mean length of 70 cycles, occurring in 15 guinea pigs under the conditions of this laboratory, was found to be 17.43 days with a probable error of the mean of $\pm 0.24$ day and a standard deviation of $\pm 3.02$ days with a probable error of the standard deviation of $\pm 0.17$ day.

As the animals grew older, however, the cycles more nearly approximated the description of Stockard and Papanicoloau; for when the same guinea pigs were studied after they had had one litter, the mean length of 30 cycles was 16.00 days with a probable error of the mean of ±0.10 day, and a standard deviation of ±0.88 day with a probable error of the standard deviation of ±0.07 day. The irregularity of the earlier cycles may have been due to the difficulty with which guinea pigs adjust themselves to strange environment and new dietary regime.

Three guinea pigs, which had had a normal oestrous rhythm upon the stock diet, were transferred to the scorbutic diet 2 with a daily protective dose (3 cc.) of orange juice. The regular occurrence of oestrus was soon resumed and maintained for about 150 days, during which the orange juice was fed successively at the levels of 3 cc., ½ cc., and ¼ cc. daily for about 50 days on each level. Table 1 shows the regularity of the oestrous rhythm even though the animals were receiving far from enough orange juice to prevent chronic scurvy. Guinea pig ♀ 26 manifested oestrus after the level of orange juice had been reduced to ⅛ cc., just before the rapid decline in growth, due to scurvy, set in. At this time the animal had painful stiff joints, and 20 days later, at autopsy, there were marked lesions of scurvy, such as general hemorrhages throughout the body, hemorrhages of the knee joints, enlargements of the costochrondral junction of the ribs, and loose molars. The ovaries contained large follicles. Guinea pig ♀ 10 was autopsied 16 days after the last oestrous period and the autopsy record was similar to that of ♀ 26. The third animal of the group, ♀ 9, after 34 days of dioestrus on ⅛ cc. of orange juice, was cured by the administration of 3 cc. orange juice daily. The oestrous rhythm was reëstablished within 10 days of the rise in growth curve.

Chart 1 illustrates the growth curve of guinea pig ♀ 9, which was typical of the group. The circles indicate the day of oestrus. Oestrus was regularly maintained throughout a period of about 100 days, during which the animal received daily only ½ cc. or less of orange juice, amounts which have been shown to permit characteristic scorbutic changes in the teeth after 14 days (Höjer, 1924).

*Loss of weight in guinea pig during oestrus.* It is apparent from the chart that while there was considerable fluctuation in the daily weight of the guinea pig, a significant loss usually occurred on the day of oestrus, which was sometimes as great as 40 grams. The loss in weight was not accompanied by any noticeable sign of malaise and was quickly regained. There was no corresponding decrease in the food consumption, such as Slonaker (1924–1925) has observed in the rat.

A loss in weight during oestrus seems to be characteristic for the guinea pig, an observation which was repeatedly confirmed in females on the stock diet, although it did not invariably occur. Rats, on the other hand, did

not change in weight at any particular time of the cycle, when they were weighed several times daily. This phenomenon may account for some

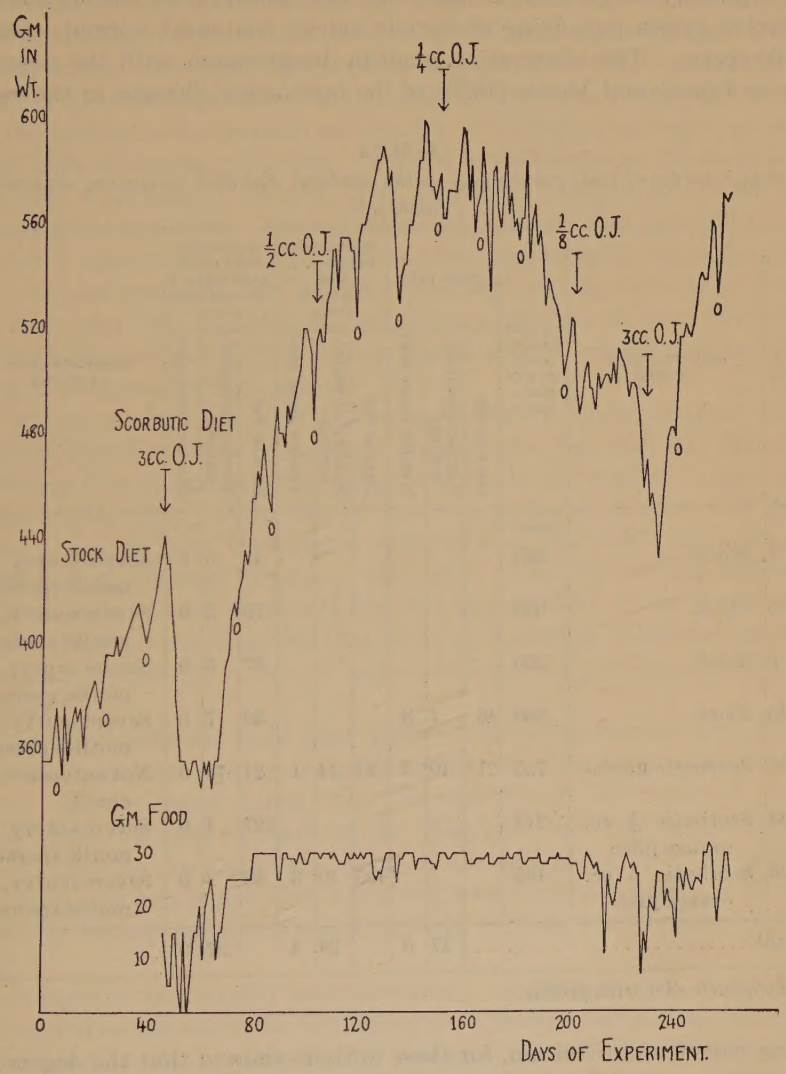


Chart 1. Growth curve, oestrous rhythm, and daily food consumption of guinea pig ♀ 9. Scorbutic diet 2 with different levels of orange juice. Letter *O* indicates oestrus.

of the large fluctuations in weight observable in guinea pigs during vitamin C tests.

*Effect of vitamin C deprivation upon the reproduction of the male guinea pig.* With regard to the influence of vitamin C deprivation upon the reproductive capability of the male, it was found that smears from the epididymis of twelve guinea pigs dying of chronic scurvy contained normal looking motile sperm. This observation is not in disagreement with the description by Linsay and Medes (1926) of the histological changes in the testis

TABLE 2

*Breeding behavior of male guinea pigs on the scorbutic diet with decreasing amounts of vitamin C*

| NUMBER OF GUINEA PIG | DIET ON WHICH REARED | WEIGHT BEGINNING OF EXPERIMENT | STOCK DIET | | | SCORBUTIC DIET NO. 2, 3 cc. ORANGE JUICE | | | SCORBUTIC DIET NO. 2 LESS THAN 3 cc. ORANGE JUICE | | | REMARKS UPON AUTOPSY |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Days on diet | Number attempted matings | Number litters sired | Days on diet | Number attempted matings | Number litters sired | Days on diet | Number attempted matings | Number litters sired | |
| | | *grams* | | | | | | | | | | |
| ♂ 1 | Stock | 350 | | | | | | | 42 | 5 | 0 | Severe scurvy, motile sperm |
| ♂ 5 | Stock | 330 | | | | | | | 73 | 3 | 0 | Severe scurvy, motile sperm |
| ♂ 6 | Stock | 300 | | | | | | | 87 | 5 | 0 | Severe scurvy, motile sperm |
| ♂ 46 | Stock | 580 | 46 | 7 | 6 | | | | 30 | 7 | 0 | Severe scurvy, motile sperm |
| ♂ 50 | Scorbutic greens | 755 | 21* | 10 | 3 | 31 | 14 | 1 | 21 | 10 | 0 | Not autopsied, cured |
| ♂ 34 | Scorbutic ½ cc. orange juice | 213 | | | | | | | 227 | 4 | 0 | Severe scurvy, motile sperm |
| ♂ 36 | Scorbutic 3 cc. orange juice | 195 | | | | 195 | 10 | 3 | 60 | 3 | 0 | Severe scurvy, motile sperm |
| Total | | | | 17 | 9 | | 24 | 4 | | 37 | 0 | |

* Scorbutic diet with greens.

during scurvy and inanition, for these authors showed that the degeneration was uneven throughout the gland and that normal tubules existed among the disintegrating ones.

As the motility of sperm is no criterion of their normal fertilizing power, some functional tests were initiated. Seven young adult males were deprived gradually of vitamin C during a period of from 1 to 8 months, by giving them scorbutic diet 2 with decreasing levels of orange juice. At frequent intervals, they were mated with normal females in oestrus. When

moribund, they were killed and autopsied, and without exception showed lesions of severe scurvy as well as motile sperm.

The females were maintained on the stock diet and were known to have had a litter either before or after the mating with the experimental animals. Vaginal smears were daily examined for plug and sperm during the mating period of two or three days, but in so few instances were they detected, that the number of litters sired per number of attempted matings was used as the index of breeding function. When 6 control males on the stock diet were bred with normal females, of 31 attempted matings, 24 (or 80 per cent) resulted in litters. The individual records varied considerably.

Table 2 summarizes the results of the breeding experiments with adult males on the scorbutic diet 2 with decreasing amounts of orange juice. The males were not mated more frequently while they were on the stock diet or on the scorbutic diet with a protective dose of orange juice, because of the limited number of females.

It will be seen from the table that not one litter was produced as the result of 37 attempted matings of males on the scorbutic diet with less than the protective dose of orange juice, a confirmation of the work of Linsay and Medes (1924–1925). With two of the same males on a protective dose (3 cc.) of orange juice, the number of litters sired was only 4 out of 24, or 16 per cent of the total number of attempts. While the mating behavior of the animals was not studied in the way described by Avery (1925) and Louttit (1927), it was observed that males on a vitamin C-low diet seemed to retain the reactions of normal males when females were introduced into the cage.

Since the functional tests were negative, it was necessary to resort to artificial insemination in order to determine the fertilizing power of the sperm. Motile sperm taken from the epididymis and testes of ♂ 6, dying of scurvy after 87 days on the vitamin C-low diet, were injected directly into the exposed uterine horns of a female in oestrus.[3] Sixty-nine days later, the female delivered a litter of three normal young. ♂ 34 and ♂ 36, the histories of which are included in table 2, were members of this litter.

One curative experiment was attempted. ♂ 50 of table 2, which had been reared on the scorbutic diet with greens, showed the typical reduction in ability to sire litters when 3 cc. of orange juice were substituted for the greens. During 33 days on a daily dose of $\frac{1}{2}$ cc. orange juice, no litter at all was sired in 10 attempts and the male lost weight and was obviously in poor condition. After the experimental period, greens were given again instead of orange juice. The animal completely recovered and in 6 months was mated with a female in oestrus, when a normal litter resulted.

[3] I wish to express my thanks to Dr. E. T. Engle of the Department of Anatomy for suggesting and performing the operation required for the artificial insemination.

It appears, then, that although males with chronic scurvy have lost the ability to sire litters, the sperm retain their motility and are capable of fertilizing the ovum. The decrease in general vigor is probably responsible for the first condition. The fact that the procreative power of males on the scorbutic diet with sufficient orange juice to insure protection against scurvy is considerably reduced, may indicate that the diet is not complete in all respects.

SUMMARY

1. The oestrous rhythm is ordinarily a sensitive indicator of the nutritional state of an animal. When guinea pigs with normal cycles were gradually deprived of vitamin C, the oestrous rhythm was found to be maintained regularly until the animal began to lose in weight. The cycles continued although the animals were receiving far from enough orange juice to protect them from chronic scurvy.

2. The onset of oestrus in the guinea pig was found to be accompanied by a sudden and temporary loss in weight, amounting sometimes to 8 per cent of the weight of the animal.

3. When males were gradually deprived of vitamin C, they lost their ability to sire litters. Smears from the epididymis of males dying of chronic scurvy contained motile sperm. That such sperm had not lost their fertilizing function was proved by artificial insemination. In a curative experiment, a male recovered the ability to sire litters.

It gives me pleasure to acknowledge my gratitude to Dr. H. T. Clarke and Dr. G. L. Foster for their kind help and encouragement.

BIBLIOGRAPHY

Avery, G. T. 1925. Journ. Comp. Psychol., v, 373.
Evans, H. M. and K. S. Bishop. 1922. Journ. Met. Res., i, 319, 335.
Höjer, A. 1924. Acta pediat., iii (suppl.).
1926. Brit. Journ. Exper. Pathol., vii, 356.
Ingier, A. 1915. Journ. Exper. Med., xxi, 525.
Linsay, B. and G. Medes. 1924–1925. Proc. Soc. Exp. Med., xxii, 177.
1926. Amer. Journ. Anat., xxxvii, 213.
Louttit, C. M. 1927. Journ. Comp. Psychol., vii, 247.
Papanicoloau, G. N. and C. R. Stockard. 1919–1920. Proc. Soc. Exp. Biol. and Med., xvii, 143.
Reyher, P., E. Walkhoff and O. Walkhoff. 1928. Münch. Med. Wochenschr., lxxv, 2087.
Sherman, H. C., U. K. La Mer and H. L. Campbell. 1922. Journ. Amer. Chem. Soc., xliv, 165.
Slonaker, J. R. 1924–1925. This Journal, lxxi, 362.
Stockard, C. R. and G. N. Papanicoloau. 1918. Amer. Journ. Anat., xxii, 225.
1919. Biol. Bull., xxxvii, 222.

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# NUTRITIONAL MUSCULAR DYSTROPHY IN THE GUINEA PIG AND RABBIT[1,2,3]

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PLATES 18 AND 19

(Received for publication, April 2, 1931)

In the course of experiments planned to study the relationship between vitamin E and reproduction in the guinea pig, it was discovered that the animals did not survive long enough on the chosen diet for breeding experiments to be carried out, and that they developed an extreme degeneration of the voluntary muscles without obvious lesions in the other organs. The diet was one composed of natural foods, in which the vitamin E had been destroyed by treatment with ethereal ferric chloride, according to the method of Waddell and Steenbock (1). However, it was later found that the addition of vitamin E to the ration did not prevent the lesions; nor was the ethereal ferric chloride treatment necessary for their production. Although the factor responsible for the muscular dystrophy has not yet been determined, we have been able to eliminate known causes of degeneration of muscle fibers, such as starvation, infection, and scurvy. A similar disorder was readily induced in rabbits by the use of the same diets, but rats appeared to be insusceptible.

While comparable lesions have been described as accompanying other disorders, a generalized and apparently primary degeneration of the entire voluntary muscular tissue has not hitherto been produced by dietary means.

[1] Some of the data in this paper are taken from a dissertation submitted by Marianne Goettsch in partial fulfilment of the requirement for the degree of Doctor of Philosophy, Columbia University, 1930.

[2] This work was aided by the Research Grant from the Chemical Foundation to the Department of Biological Chemistry.

[3] A preliminary report was presented at the March, 1930, Meeting of the Society for Experimental Biology and Medicine.

### *Preparation of the Diet*

The diet was prepared as follows:

*Diet 13*

| | |
|---|---|
| Rolled oats (Quaker) | 355 parts |
| Wheat bran (Pillsbury) | 180 " |
| Casein (Merck technical) | 75 " |
| Lard | 80 " |
| Cod liver oil (Mead, Johnson) | 10 " |
| NaCl | 10 " |
| $CaCO_3$ | 15 " |

10 gm. of ferric chloride, U.S.P. lump, was taken up in about 125 cc. of ether and a little water, and the solution poured over the above ingredients. The mass was shaken in a closed container and allowed to stand for about half an hour. The contents were emptied upon a tray and the ether allowed to evaporate, usually overnight. Finally there was added:

Skimmed milk powder (Merrell-Soule)—275 parts.

Diet 11 was similar to Diet 13 except that the treatment with ethereal ferric chloride was omitted. The diets were freshly prepared every 2 to 3 days and were always consumed within 7 days. Diet 13 possessed an acrid odor as described by Waddell and Steenbock (1) in their vitamin E-deficient diet.

Each guinea pig was given daily by pipette 3 or 6 cc. of orange juice.

Diet 13 was calculated to contain 3.7 per cent of roughage, to have a nutritive ratio of 1:3.6, with two-thirds of the proteins supplied by milk products, and a calcium-phosphorus ratio of 1.62:1. Vitamins A and D were supplied by the cod liver oil, B and G by the grain and milk products, and C by the orange juice. The vitamin E content was purposely reduced to a minimum. The description of control experiments with Diet 13 carried out upon rats will follow in a later section of the paper.

### *Effect of Diet 13 upon Guinea Pigs*

Guinea pigs that had been born in the laboratory were used in the present experiments, and upon weaning at 21 days, they were given Diet 13 with lettuce *ad lib.* and orange juice. After about 10 days, when they had become more or less accustomed to the new diet, the greens were discontinued. Other groups of guinea pigs were given either Diet 11, or a stock diet of oats, bran, and greens.

The rate of growth for the first month or two was good for all groups. After the animals had attained a weight of about 400 gm., there was a sudden cessation in the growth of the animals on Diet 13; this was accompanied by general flabbiness, and it was observed that the guinea pigs had difficulty in righting themselves if they were placed

on their back. During the following month, a constant weight was usually maintained, after which the guinea pigs went into rapid decline for 2 or 3 days and suddenly died. A few of the animals recovered spontaneously from the flabbiness, made a moderate gain in weight, remained stationary at a higher level, and died. The terminal loss in weight was often as great as 15 to 20 per cent of the maintenance weight.

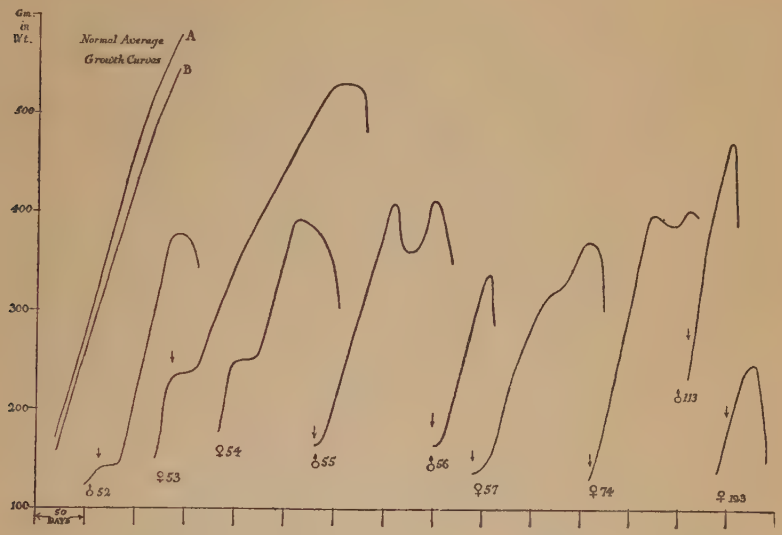


CHART 1. Typical growth curves of guinea pigs on Diet 13 and 3 or 6 cc. of orange juice daily.

*A*. Composite growth curve of 770 normal male guinea pigs from data of Wright (2).

*B*. Composite growth curve of three males and four females on the stock diet of oats, bran, and greens.

The average survival period was about 90 days but there was great individual variation, as is shown in Table I. Two guinea pigs died after only 35 and 37 days on the diet, while two others lived for 186 and 218 days. Litter mates were found to vary as much as others in this respect. The group on stock diet showed no break in the growth curve and continued to increase in weight steadily.

Typical growth curves of guinea pigs on Diet 13 are illustrated in Chart 1. In Chart 2, the growth curve of Female 53 is shown in de-

tail. This guinea pig lived for 186 days on Diet 13. Estrus, indicated by the letter E, is seen to have occurred regularly at the normal intervals of 16 to 18 days, (Stockard and Papanicolaou (3)) even when

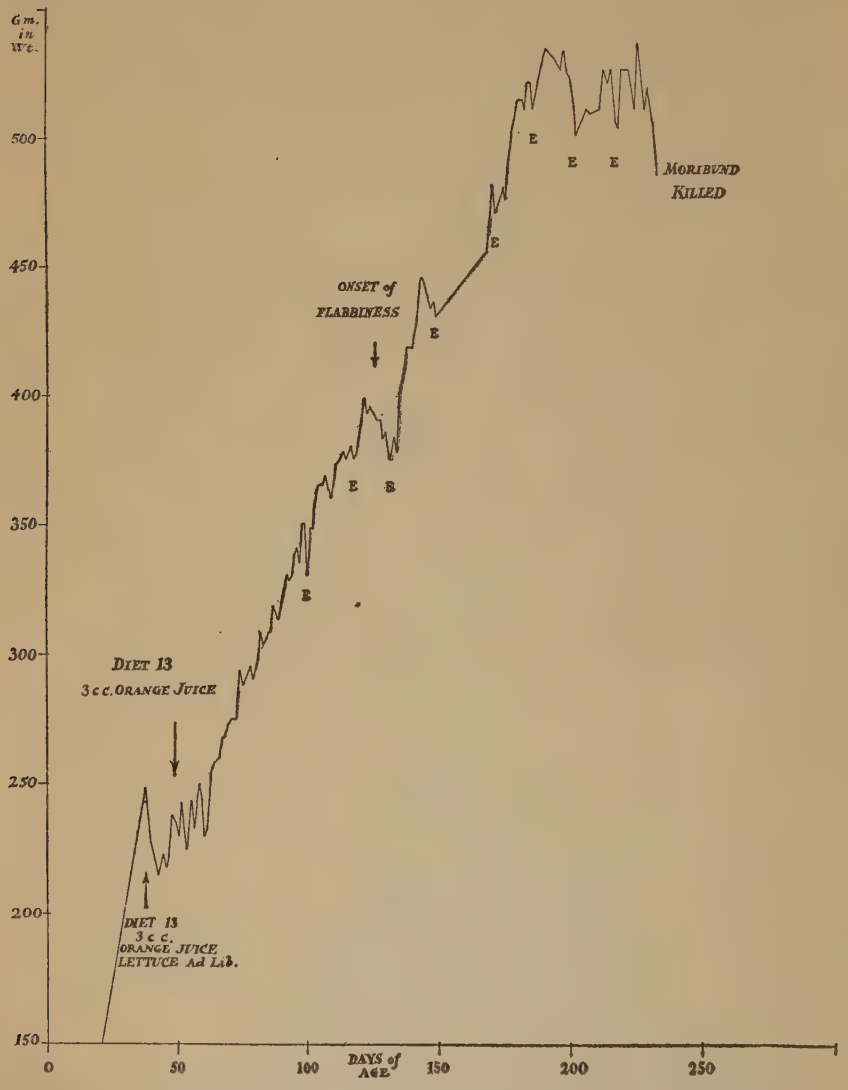


CHART 2. Growth curve and estrous rhythm of Guinea Pig ♀ 53 on Diet 13 and 3 cc. of orange juice daily. Letter E indicates estrus.

the animal had begun to show definite symptoms. The estrous rhythm was generally normal in the guinea pigs on Diet 13 as well as on Diet 11. Further studies upon reproduction were impossible on account of the early death of the animals. Males reared on Diet 13 showed, at autopsy, normal-looking motile sperm in smears from the epididymis, although one had been on the diet as long as 218 days.

### *Pathological Changes*

*Gross Appearances.*—While most of the animals were killed and autopsied when apparently on the point of death, a few were sacrificed before the cessation of growth. Of the 30 experimental guinea pigs, all but three showed striking macroscopic changes in the skeletal muscles. The muscles of the thigh and abdomen were particularly abnormal, although the muscles of the back and the extremities, the diaphragm, and the intercostal muscles were involved. They were atrophied, pale, with a yellowish or yellowish brown color, and less translucent than those of the controls. Sometimes they appeared gritty and streaked as though calcified or infiltrated with fat. In addition to their abnormal appearance, the muscles had lost their irritability to mechanical stimulation and failed to contract when severed.

In contrast to the wasting of the skeletal muscles, the subcutaneous adipose tissue and that within the peritoneal cavity were often strikingly abundant, an indication that the fall in weight was at the expense of the muscle rather than of the fat. No gross changes of significance were noted in the internal organs. The heart was normal. The lungs in some animals showed areas of pneumonic consolidation, in others were well aerated and of normal appearance. The liver in the majority of animals showed fat infiltration. The spleen was small, firm, and brownish. The kidneys, adrenals, genitalia, and gastrointestinal tract were normal.

*Microscopic Lesions.*—The pathological changes of significance were restricted to the striated skeletal muscles of the trunk and extremities, in which the lesions were profound, widespread, and characteristic. In guinea pigs, practically every voluntary muscle, including the diaphragm, was affected to a varying degree with the exception of the masseter and tongue muscles, which preserved their integrity to

the end. The muscles examined as routine were the quadriceps femoris, the adductors of the thigh, the pectorals, the abdominal muscles, the diaphragm, and the masseter. At various times, blocks were taken from many other muscles as well.

The histological changes depend somewhat upon the stage of the disease, but the essential and primary lesion is a coagulative necrosis of the muscle fiber, presenting the familiar picture of waxy, hyaline, or Zenker's degeneration (Fig. 1). This is followed by reactive cellular multiplication with attempts at regeneration. Many of the necrotic fibers become resorbed and their place taken by fat and connective tissue. The late stages, as will be shown, resemble closely the changes seen in progressive muscular dystrophy of man.

While all the muscles examined showed these degenerative lesions to a greater or lesser degree, the muscles were not always uniformly affected, certain bundles being but slightly involved, while in others almost 100 per cent of the fibers were necrotic.

The earliest change in the fiber is shown in the appearance of transverse ridges or contraction bands, in which the striations are brough more closely together. The discs become disarranged and lose their individual identity to fuse into a swollen hyaline mass. Fibers cut in their long axis may show a succession of more or less globular hyaline masses (Fig. 1), between which the sarcolemma is collapsed, containing only granular detritus. The sarcolemma nuclei are displaced and distorted, and tend to become oriented at right angles to the long axis of the fiber; subsequently they undergo karyorrhexis and may disappear completely.

Preparations from many of the animals were obtained in this stage, before any marked cellular reaction had occurred. In most instances, however, side by side with the necrobiotic changes, there was found an active multiplication of cellular elements with numerous mitoses. The cells have deeply staining oval nuclei, and a rather small amount of purplish cytoplasm. They lie in clefts in the coagulated and necrotic muscle substance, and may completely fill the original sarcolemma sheath. The cylindrical columns of closely packed cells constitute the "*Muskelzellenschlaüche*" of Waldeyer (Fig. 2). The origin of these cells, whether from invading histiocytes or from the division of the uninjured muscle nuclei with their surrounding sarcoplasm, has been often discussed. Reference may be made to the excellent studies of Forbus (4), who by means of preliminary vital staining, was able to distinguish the invading phagocytic histiocytes, which aid presumably in the removal of the necrotic material, from the proliferating muscle nuclei, which are concerned in the regeneration of new fibers.

It has seemed to us that the majority of the mononuclear cells making up the "*Muskelzellenschlaüche*" were derivatives of the muscle cells themselves, although we have occasionally observed the invasion of the dead fibers by polymorphonuclear leucocytes (Fig. 3). Many of them, as Forbus and others have pointed out, undergo subsequent degeneration, but others assume a spindle shape, align them-

selves in rows, and by the development of myofibrils on their surface, give origin to regenerating muscle fibers (Fig. 4). Because of their basophilic staining, such young regenerating fibers are easily distinguished from the persistent original fibers which have escaped destruction. In many of our preparations, there is a conspicuous formation of large multinucleated plasmatic masses, lying against the necrotic remains of the muscle substance (Fig. 5). Calcification of fibers was occasionally seen (Fig. 6).

It has been stated that in the more chronic cases, only occasional necrotic fibers were to be seen, but the remaining fibers were often atrophic and widely separated by strands of cellular connective tissue or by fat. These muscles with replacement fibrosis and lipomatosis closely resemble those of progressive muscular dystrophy in man (Fig. 7).

The pathological changes described above are definitely restricted to the striated skeletal muscles. The myocardium and the smooth muscle fibers of the intestine, bronchi, blood vessels, and uterus are entirely unaffected.

As regards the question whether the degeneration of the muscles is primary, or secondary to alterations in the nervous system, it may be said that the spinal cord and peripheral nerves show no significant changes. While the nervous tissues have not been systematically examined in all cases, sections have been made in sufficient number to show clearly that the disease of the muscles is not associated with degeneration of the ganglion cells of the central nervous system nor of the peripheral nerve trunks. Even in muscles showing extreme destruction of the great majority of the fibers, the small intermuscular nerves present no recognizable alteration.[4] There still remains the possibility that the first injury falls upon the motor end plates; this needs further investigation.

That the muscle lesions may develop very rapidly is indicated by the following observations:

Guinea Pig 101, male. Born Dec. 16, 1929. Weaned Jan. 12, 1930. Diet 13 plus 6 cc. of orange juice started on Jan. 22, 1930. On Mar. 24th, after having been 61 days on the diet, a piece of adductor muscle was excised from the right thigh. Two blocks examined showed practically no abnormal fibers: near the edge

[4] We are indebted to Dr. W. M. Rogers of the Department of Anatomy and to Dr. Abner Wolf of the Department of Pathology for silver preparations of our material which demonstrate the integrity of the terminal nerves in severely degenerated muscles.

of the section was a single muscle cell column. On the 75th day, at which time the decline in weight had begun and distinct symptoms of muscular weakness had become manifest, another muscle specimen was excised from the left adductor. The lesions were now marked: there were numerous necrotic fibers and much cell proliferation.

The animal died on the following day, and was found to have most intense lesions of all the muscles examined.

In this animal, therefore, there is evidence that the alterations in the muscle lesions occurred only during the last 2 weeks of life.

In several other animals, the progress of the disease was followed by removing pieces of muscle for microscopic study at various times after beginning the experimental diet. The rather abrupt onset of the muscle lesions was again noted. Thus in Guinea Pig 107, specimens obtained on the 36th and 49th days showed no lesions. On the 65th day, there were occasional necrotic fibers, with moderate multiplication of the muscle nuclei. On the 78th day, the lesions were very marked. There were numerous necrotic and segmented fibers, and great proliferation of the muscle nuclei. The animal died on the 108th day and showed advanced changes, with much fibrous and fatty replacement of the degenerating muscles.

*Microscopic Changes in Other Organs and Tissues.*—The myocardium was unaltered. The lungs, in a large proportion of the guinea pigs and rabbits were the seat of a bronchopneumonia of varying extent, often associated with areas of atelectasis. In a number of the animals, the lungs were normal. The liver frequently exhibited a moderate degree of fat infiltration. The spleen was small and brownish; it contained hemosiderin deposits in the pulp in definitely increased amount.

None of the other viscera, including pancreas, adrenals, kidneys, gonads showed changes to which any importance could be attached. As might be anticipated, the deficiency in vitamin E did not inhibit spermatogenesis, the duration of the survival period being too short for this.

## *Summary*

A general oversight of our material is given in Table I. Of thirty guinea pigs on Diet 13, eight were not studied microscopically; however, six of these presented the symptoms and gross changes which were later recognized as characteristic and the remaining two manifested symptoms, but no gross lesions were noted. The remaining twenty-two showed characteristic microscopic changes.

### *Effect of Diet 13 upon Rabbits*

Two rabbits, Nos. 1 and 3, born in the laboratory, were placed upon Diet 13 with 6 cc. of orange juice daily, when they were 4½ weeks of age. They maintained

TABLE I

*Showing the Effect of Diet 13 upon Guinea Pigs*

| Guinea pig | Days on diet | Extent of muscle lesion | | Lung infection | Remarks |
|---|---|---|---|---|---|
| | | Gross | Microscopic | | |
| ♂ 52 | 76 | Normal | Not examined | +++ | Moribund, killed |
| ♀ 53 | 186 | ++++ | ++++ | + | " " |
| ♀ 54 | 97 | ++ | ++ | Normal | " " |
| ♂ 55 | 119 | ++ | Not examined | " | " " |
| ♂ 56 | 48 | ++ | " " | + | " " |
| ♀ 57 | 122 | ++ | ++ | Normal | " " |
| ♀ 58 | 122 | ++ | +++ | + | " " |
| ♂ 59 | 133 | ++ | +++ | Normal | " " |
| ♀ 60 | 77 | +++ | Not examined | +++ | " " |
| ♀ 61 | 110 | ++ | " " | + | Died |
| ♀ 62 | 134 | ++ | ++++ | Normal | Moribund, killed |
| ♀ 63 | 92 | +++ | Not examined | " | Died |
| ♂ 68 | 63 | +++ | +++ | + | " , advanced p.m. changes |
| ♀ 72 | 72 | ++ | ++ | Normal | Moribund, killed |
| ♀ 74 | 98 | + | ++ | ? | Biopsy |
| ♀ 80 | 58 | +++ | +++ | + | Moribund, killed |
| ♀ 81 | 55 | ++ | ++++ | Normal | " " |
| ♀ 85* | 58 | ++ | +++ | + | " " |
| ♂ 86* | 35 | + | ++++ | Normal | " " |
| ♂ 93 | 40 | Normal | Not examined | " | Died, advanced p.m. changes |
| ♂ 101 | 77 | +++ | ++++ | + | " |
| ♂ 104 | 24 | + | Not examined | +++ | " , advanced p.m. changes |
| ♂ 107 | 85 | + | +++ | Normal | " |
| ♂ 109 | 218 | ++++ | ++++ | " | Moribund, killed |
| ♀ 110 | 131 | + | ++++ | " | Died, advanced p.m. changes |
| ♂ 113 | 51 | +++ | ++++ | ++ | Moribund, killed |
| ♀ 115 | 137 | +++ | ++ | Normal | " " |
| ♂ 116 | 76 | ++ | +++ | + | " " |
| ♂ 119 | 133 | ++ | ++ | + | " " |
| ♀ 123 | 37 | +++ | ++++ | Normal | " " |

* Whole wheat substituted for bran in Diet 13.

their weight and even grew to some extent for 12 days, then lost the use of their muscles completely, and were killed and autopsied 2 and 4 days later. The muscles

were small, extremely pale, and without irritability. Five older rabbits, weighing from 385 to 730 gm., were also given Diet 13 with orange juice. They survived for a longer time, but after 24 to 50 days became prostrated and upon autopsy showed marked macroscopic changes in the voluntary muscles. The other organs apparently were normal.

The muscle lesions in rabbits are essentially the same as those in guinea pigs. They are in general more rapid in their development; extreme lesions have been noted as early as 15 days after the institution of the diet. The masseter shares in the general muscular degeneration, and there is greater tendency for the necrotic fibers to become

TABLE II

*Showing the Effect of Diet 13 upon Rabbits*

| Rabbit | Weight at beginning of experiment | Days on diet | Extent of muscle lesion | | Lung infection | Remarks |
|---|---|---|---|---|---|---|
| | | | Gross | Microscopic | | |
| | *gm.* | | | | | |
| ♀ 1 | 335 | 14 | +++ | ++++ | + | Moribund, killed |
| ♀ 3 | 340 | 16 | +++ | ++++ | + | " " |
| 4 | 450 | 5 | Normal | + | Normal | Died |
| 5 | 425 | 24 | ++ | +++ | +++ | Moribund, killed |
| ♂ 10 | 730 | 50 | ++ | ++ | ++ | Died |
| 11 | 490 | 27 | Normal | Slides lost | +++ | Moribund, killed |
| 12 | 385 | 27 | + | +++ | +++ | Died |

calcified. We have noted no other differences in reaction between rabbits and guinea pigs.

The experiments with rabbits are summarized in Table II.

### *Failure of Rats to Develop Muscular Dystrophy on Diet 13*

Rats reared on Diet 13 gave no symptoms of the muscular dystrophy characteristic of guinea pigs and rabbits, although they were maintained on the diet for more than a year. Some of the young from mothers on Diet 13 were continued on the diet until they were old enough to be bred. They are listed as second generation in Table III. Young from second generation rats were likewise continued on the diet and formed the third generation. A detailed microscopic study of muscles from a second generation rat, killed at 30 days, revealed an

occasional necrotic fiber, while three stock controls of the same age were normal. Two third generation rats, young from B361 of Table III, were killed at 4 months and were found to have muscles that were entirely normal.

### *Discussion of Possible Factors*

Before attributing these widespread changes in the muscular system to any specific characteristics in the diet, one should exclude, if possible, certain well recognized causes of muscular degeneration and these may be briefly discussed.

*1. Starvation.*—In the terminal stage of the muscular dystrophy there is, as has been shown, a rapid loss of weight. With the increasing muscular paresis, there is often inability to take food. It has been shown by Kremer (5), that long continued fasting in the hibernating frog leads to extreme muscular necrobiosis, associated with excessive multiplication of muscle nuclei. The illustrations in Kremer's article can be duplicated in our material. While a few days of partial inanition should not be expected to result in comparable change, it was thought wise to control this possibility by a limited number of starvation experiments.

An adult guinea pig, No. 1, was given only water and 6 cc. of orange juice daily for a period of 12 days, during which the weight declined from 1060 to 840 gm. Sections of masseter, quadriceps femoris, pectoralis, diaphragm, and adductors of the thigh showed complete absence of degenerative or necrobiotic changes.

Guinea Pig 31 was fasted for 11 days under similar conditions, the weight declining from 880 to 715 gm. Again no significant changes were found in the voluntary muscles.

It seems evident, then, that in spite of the rapid loss of weight, amounting to 18 and 21 per cent of the original body weight, the muscular lesions cannot be ascribed merely to inanition. The preservation of the adipose tissue even in cases showing extreme muscular degeneration is additional evidence that we are not dealing merely with the effect of inanition.

*2. Infection.*—All the rabbits and half the guinea pigs died with a terminal bronchopneumonia. Whether the powdery diet, unnatural for animals normally subsisting on coarse vegetable food, was a contributory factor, or whether the weakness of the diaphragm and intercostals also predisposed, cannot be stated. Zenker's degeneration, especially of the rectus abdominis, but also of other muscles, is a common accompaniment of typhoid fever, pneumonia, and influenza in man. The question therefore must be answered as to whether the muscle changes in the experimental animals were related to the pneumonia or other terminal infection.

This possibility can be very definitely excluded. Many of the guinea pigs in this and subsequent experiments (see Table I) and Rabbits 15 (Table IV) and 19

(Table V) died with normal lungs, and without any indication of infection in other organs. Pieces of muscle have been excised and found to show lesions at a time when there was little or no loss of weight or other evidence of illness. Furthermore, we have studied as control, muscles from several rabbits with experimental pneumococcus pneumonia, or with general pneumococcus septicemia,[5] and have found no similar lesions.

*3. Scurvy.*—Inasmuch as several workers, notably Höjer (6), Meyer and McCormick (7), Dalldorf (8) have reported the occurrence of hyaline necrosis of the muscle fibers in the experimental scurvy of guinea pigs, it seemed highly important to be certain that our animals were not suffering from latent scurvy. Sherman (9), using as criterion of scurvy looseness of teeth, fragility of bones, enlargements and hemorrhages of joints and rib junctions, obtained complete protection with a daily addition of 1.5 cc. of orange juice for a period of 90 days. Höjer (10) devised a method for assaying vitamin C based upon the microscopic alterations in the odontoblasts and dentine of the incisor root after 10 to 14 days on the diet. Goettsch and Key (11), comparing this test with Sherman's, found that 3 cc. of orange juice daily was needed for complete protection, and Eddy (12), using the same technique, arrived at a similar quantitative result. Dalldorf and Zall (13) found slight scorbutic lesions in the incisor roots with 5 cc. Using the rate of tooth growth as a test, they found that 5 cc. was not quite adequate, and that "for short periods and conservative diagnosis, 7 cc. might be considered protective."[6]

On the basis of the evidence of previous workers, it might be assumed that the addition of 3 or 6 cc. of orange juice daily should protect against the gross manifestations of scurvy, although it might not give complete protection if the microscopic alterations of the teeth or their rate of growth are taken as adequate criteria. The generally recognized evidences of scurvy were not present in our animals. Hemorrhages were never seen, either in the vicinity of the joints or elsewhere, even in animals showing the most advanced and universal muscle changes. Microscopic section of the ribs showed not the slightest evidence of scurvy, osteogenesis being active, the marrow free from fibrotic changes, and hemorrhages absent. While the teeth have not been studied in all cases, sections from animals with advanced muscular lesions have shown in a number of instances (Fig. 8), normal incisor roots.[7] Finally, the production of identical muscular changes in rabbits, which are notoriously resistant to experimental scurvy, makes it highly improbable that the muscular lesions are due to a deficiency in vitamin C.

*4. Possible Deficiencies of Diet 13 in Other Vitamins. Vitamin A.*—That the vitamin A content of Diet 13 was not destroyed by the ethereal ferric chloride

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[5] We are indebted to Mrs. Frances M. Weld for placing these animals at our disposal.

[6] Personal communication from Dr. Dalldorf.

[7] We wish to express our thanks to Professor Charles Bödecker of the School of Dentistry, for preparing sections of the teeth.

treatment, was proved: first, by the fact that histological sections of epithelial tissue from guinea pigs reared on the diet revealed none of the keratinization described by Mori (14) and Wolbach and Howe (15), as typical of vitamin A deficiency; and secondly, by the failure of rats reared on the diet to show in their estrous cycles, the prolonged period of "cornified" cells shown by Evans and Bishop (16) to be associated with lack of vitamin A.

TABLE III

*The Effect of Diet 13 on Female Rats*

Showing the occurrence of resorptions, typical of vitamin E deficiency, and their cure by the administration of substances containing E.

| Rat | Generation | 1st gestation | | 2nd gestation | | Gestation following resorption | | |
|---|---|---|---|---|---|---|---|---|
| | | R.B.C.* | No. of young | R.B.C. | No. of young | Curative substance | R.B.C. | No. of young |
| W47 | 1st | + | 5 | + | 0 | — | | |
| W57 | 1st | + | 0 | | | Diet 11 | + | 8 |
| W64 | 1st | + | 2 | + | 0 | " 11 | + | 9 |
| W65 | 1st | + | 6 | + | 0 | — | | |
| W66 | 1st | + | 2 | + | 0 | Diet 11 | + | 9 |
| W233 | 1st | + | 1 (dead) | + | 0 | — | | |
| W281 | 1st | + | 5 | + | 4 (2 dead) | | | |
| BH298 | 1st | + | 6 | + | 0 | 550 mg. W.G.O. | + | 0 |
| W350 | 1st | + | 0 | | | 550 " " | + | 9 |
| B361 | 2nd | + | 5 | + | 0 | 550 " " | + | 0 |
| B362 | 2nd | + | 5 | + | 0 | 550 " " | + | 5 |
| B363 | 2nd | + | 4 (1 dead) | + | 0 | 550 " " | + | 5 |
| B364 | 2nd | Never mated | | | | | | |
| B365 | 2nd | " " | | | | | | |
| W366 | 2nd | + | 0 | | | — | | |

* R.B.C. + indicates successful implantation.

*Vitamin D.*—That there was sufficient vitamin D in the diet was indicated by the fact that neither rabbits nor rats, including second generation animals, showed rickets in the course of these experiments. Rickets has never been observed in guinea pigs.

*Vitamins B and G.*—The amount of vitamins B and G necessary for guinea pigs and rabbits is not known. However, since the diet contained an adequate amount of B and G for the rat, and since the guinea pigs and rabbits were free from any evidences of polyneuritis or dermatitis, it seems improbable that a deficiency in these factors entered into the problem.

### *The Deficiency in Vitamin E*

That Diet 13 was deficient in vitamin E content, as planned, was proved by the following experiments with rats:

Rats both male and female, when reared upon Diet 13, responded to the technique of Evans and Burr (17) in a way typical of vitamin E deficiency. Five males after an initial period of fertility, were unable to sire litters after 200 to 250 days on the diet. Upon autopsy, after 230 days, two functionally sterile males showed, in smears from the epididymis, histologically normal spermatozoa, a few of which were motile. In smears from the other three males after 420 days on the diet, there were found either no sperm at all, or many dead atypical ones. Three litter mate control rats upon untreated Diet 11 retained their fertility as judged by functional tests, and at 420 days had normal motile sperm.

Table III shows the reproductive history of thirteen female rats on Diet 13, that were bred at the age of 3 months. Three rats had typical resorption of the embryos during the first gestation, nine had "first litters" followed by resorptions in subsequent pregnancies, and one had two litters. Following resorptions, cures were attempted with Diet 11 as well as with a known source of vitamin E, wheat germ oil. As is shown in Table III, three females were successfully cured by giving them Diet 11 throughout the trial gestation period and three of five females were cured by the administration of 550 mg. of wheat germ oil on the day of positive mating.

Thirty young rats from mothers that had been reared and maintained on Diet 13 during gestation and lactation, did not suffer from the paralysis sometimes shown by young from mothers on a vitamin E-deficient diet during lactation (Evans and Burr (18)).

### *Elimination of Vitamin E as a Factor in the Production of Muscular Dystrophy*

Since Diet 13 was originally planned and subsequently proved to be deficient in vitamin E, it was necessary to ascertain whether the muscle lesions in guinea pigs and rabbits might be a hitherto unrecognized effect of a deprivation in this factor. This question was approached in three ways: (1) by supplementing Diet 13 with vitamin E in the form of tested wheat germ oil, (2) by placing the animals on a vitamin E-low diet made up of simplified foods, such as was used by Evans and Bishop in their experiments upon rats, and (3) by giving the Diet 13 without ferric chloride treatment (Diet 11). The last experiment also served to test the possibility that the ferric chloride *per se* had a deleterious effect upon the muscle, or that it acted destructively upon some unknown necessary constituent in the experi-

mental diet, or that its presence in the diet resulted in the formation of some substance toxic for the muscle tissue.

*1. Addition of Wheat Germ Oil to Diet 13.*—Ten guinea pigs were given Diet 13 plus a daily supplement, administered by pipette, of 6 cc. of orange juice and 8 drops (approximately 200 mg.) of wheat germ oil. This wheat germ oil was assayed for vitamin E content according to the technique of Evans and Burr (17). When given on the day of positive mating, in a single dose of 550 mg. (a quantity equivalent to 1 drop of oil a day for 22 days), it usually cured female

TABLE IV

*Showing the Effect of the Addition of Wheat Germ Oil to Diet 13 upon Guinea Pigs and Rabbits*

| No. of animal | Weight at beginning of experiment | Daily dose of W.G.O. | Days on diet | Extent of muscle lesion | | Lung infection | Remarks |
|---|---|---|---|---|---|---|---|
| | | | | Gross | Microscopic | | |
| | *gm.* | *mg.* | | | | | |
| G. P. ♀ 94 | | 200 | 31 | Normal | Normal | +++ | Killed |
| G. P. ♂ 114 | | 200 | 35 | + | " | + | Moribund, killed |
| G. P. ♂ 106 | | 200 | 46 | Normal | " | + | Died |
| G. P. ♀ 111 | | 200 | 50 | " | " | Normal | Moribund, killed |
| G. P. ♀ 130 | | 200 | 48 | +++ | ++++ | " | " " |
| G. P. ♀ 103 | | 200 | 59 | Normal | ± | " | Died |
| G. P. ♂ 108 | | 200 | 75 | " | ± | " | " |
| G. P. ♀ 97 | | 200 | 122 | " | ++ | " | " |
| G. P. ♀ 96 | | 200 | 139 | + | +++ | + | " |
| G. P. ♀ 112 | | 200 | 233 | + | ++ | Normal | Moribund, killed |
| R. 6 | 415 | 450 | 45 | ++ | ++++ | +++ | Died |
| R. 8 | 420 | 450 | 32 | + | +++ | +++ | Moribund, killed |
| R. ♂ 13 | 630 | 450 | 56 | +++ | +++ | +++ | " " |
| R. ♀ 14 | 540 | 450 | 53 | +++ | +++ | + | " " |
| R. ♂ 15 | 490 | 450 | 55 | +++ | ++++ | Normal | " " |

rats that had been proved to have resorptions. Cures were invariably obtained with single doses of 1.1 gm. (an amount equal to 2 drops of oil a day). The guinea pigs, therefore, received from four to eight times as much wheat germ oil daily as was found necessary to cure the rats, although their body weight was only two to three times as great; and furthermore, the oil was administered from the day of weaning, so that the total amount given was in considerable excess of that required by rats.

As is shown in Table IV, of the ten guinea pigs on the vitamin supplemented Diet 13, four did not survive very long and showed normal muscles at death.

Guinea Pigs 103 and 108, dying on the 59th and 75th days, had in sections, occasional necrotic fibers with little cellular reaction. The other four guinea pigs, which lived from 48 to 233 days, presented very striking and widespread muscular lesions.

Chart 3 illustrates not only the similarity of the growth curves of animals of this group to those of animals showing characteristic muscle dystrophy on Diet 13, but also the great individual variation among them.

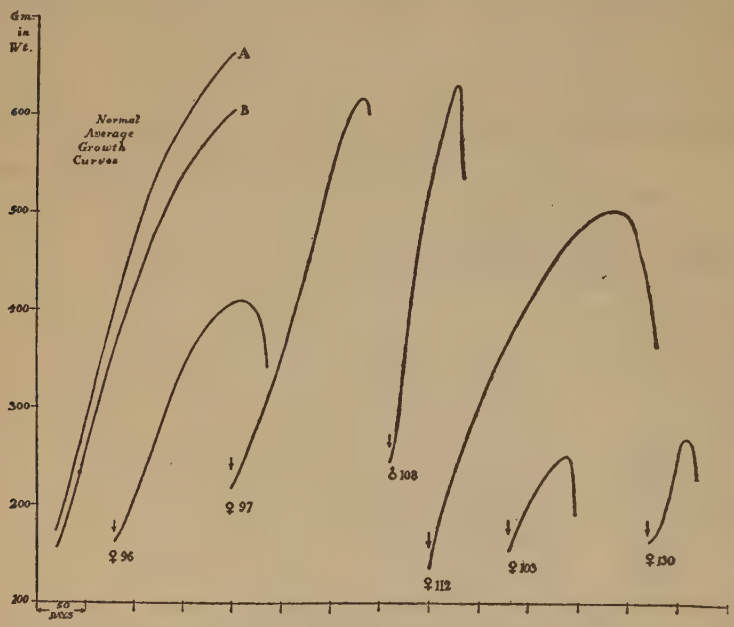



CHART 3. Growth curves of guinea pigs on Diet 13 with the daily supplement of 6 cc. of orange juice and 200 mg. (8 drops) of wheat germ oil.

*A*. Composite growth of 770 normal male guinea pigs from data of Wright (2).

*B*. Composite growth curve of three males and four females on the stock diet of oats, bran, and greens.

Table IV also includes data from five rabbits on Diet 13 plus 6 cc. of orange juice and 450 mg. of wheat germ oil. Without exception, they exhibited characteristic symptoms and severe gross and microscopic lesions in the muscles, in the same length of time as the group without the wheat germ oil supplement.

*2. Effect of a Simplified Food Diet with and without Vitamin E upon Guinea Pigs.*—An attempt was made to study the possible rôle of vitamin E deficiency in the development of the muscular dystrophy by placing guinea pigs upon a simplified diet deficient in this factor. The diet consisted of 27 per cent casein, 59 per cent

corn-starch, 8 per cent lard, 4 per cent McCollum salt Mixture 185 (19), 8 per cent Fleischmann's bakers' dried yeast, and 2 per cent cod liver oil, and was found to be adequate for rats except in its vitamin E content.

When this diet with 6 cc. of orange juice was given to fourteen young guinea pigs, half of them altogether refused to eat it, and the other seven made very poor gains in weight and were in an evident state of malnutrition. Five survived for only 11 to 45 days and had normal muscles at autopsy. Of the remaining two, No. 89 lived for 139 days. It never exceeded a weight of 275 gm. At autopsy

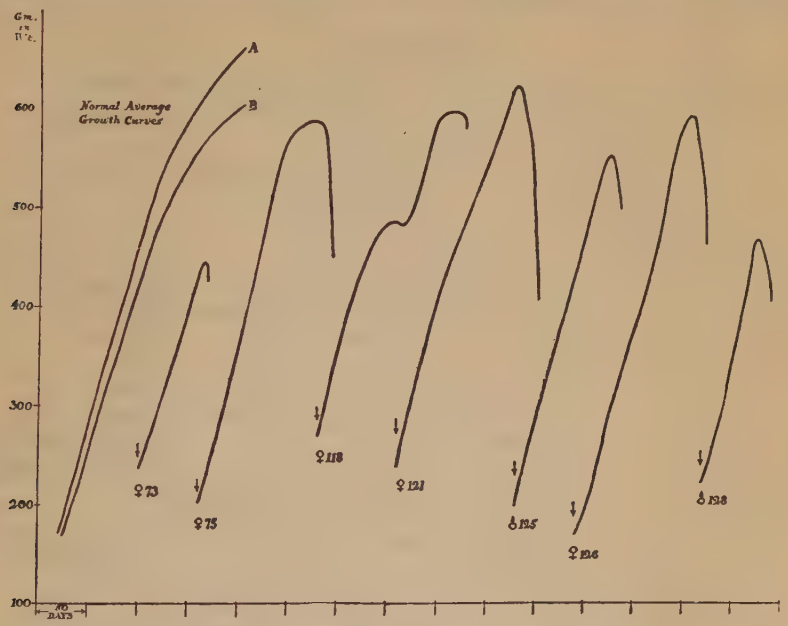



CHART 4. Growth curves of guinea pigs on Diet 11 and 3 or 6 cc. of orange juice daily.

*A*. Composite growth curve of 770 normal male guinea pigs from data of Wright (2).

*B*. Composite growth cu ve of three males and four females on the stock diet of oats, bran, and greens.

the muscles were reduced in bulk, red, dry, and not very contractile. Microscopically there was great atrophy with fibrous replacement; degenerating fibers were still numerous. The masseter as usual was relatively normal. The picture was identical with that seen on Diet 13, when the animal survived for a corresponding period. The other animal, No. 92, had been given 200 mg. of wheat germ oil in addition to the simplified diet and orange juice. It survived for 318 days, reaching a maximum weight of 440 gm. The muscles at autopsy showed only occasional necrotic and calcified fibers.

The unsuitability of this diet for maintaining the guinea pigs in a satisfactory state of nutrition did not justify further experimentation.

*3. Effect of Untreated Diet 11 upon Guinea Pigs and Rabbits.*—Litter mates of the guinea pigs on Diet 13 were maintained on the untreated Diet 11 with 3 or 6 cc. of orange juice a day. As shown in Chart 4, they grew at a normal rate for a longer period of time than the animals on Diet 13, most of them attaining a weight of 550 gm. In the earlier experiments, the controls were actively growing and apparently in excellent condition at the time that their litter mates were dying

TABLE V

*Showing the Effect of Diet 11 upon Guinea Pigs and Rabbits*

| No. of animal | Weight at beginning of experiment | Days on diet | Extent of muscle lesion | | Lung infection | Remarks |
|---|---|---|---|---|---|---|
| | | | Gross | Microscopic | | |
| | *gm.* | | | | | |
| G. P. ♂ 84* | | 38 | Normal | Normal | ? | Biopsy |
| G. P. ♂ 79 | | 58 | " | Not examined | Normal | Killed |
| G. P. ♂ 82* | | 62 | " | Normal | ? | Biopsy |
| G. P. ♀ 87* | | 61 | " | " | ? | " |
| G. P. ♀ 73 | | 75 | " | " | + | Killed |
| G. P. ♀ 75 | | 141 | ++ | +++ | Normal | Died |
| G. P. ♀ 118 | | 165 | Normal | + | ? | Biopsy, still alive |
| G. P. ♀ 121 | | 145 | ++ | ++++ | + | Moribund, killed |
| G. P. ♂ 125 | | 107 | Normal | ++++ | Hemorrhage | Died |
| G. P. ♀ 126 | | 133 | ++ | ++++ | Normal | " |
| G. P. ♂ 128 | | 87 | +++ | ++++ | " | Moribund, killed |
| R. 7 | 475 | 30 | Normal | Normal | " | Killed |
| R. 9 | 340 | 32 | " | + | +++ | " |
| R. ♀ 19 | 610 | 59 | +++ | +++ | Normal | Died |
| R. ♂ 20 | 590 | 56 | +++ | ++++ | ++ | Moribund, killed |
| R. ♂ 21 | 500 | 56 | +++ | +++ | ++ | Died |

* Whole wheat substituted for bran in Diet 11.

of the muscular dystrophy. Upon microscopic examination during this stage their muscles revealed no lesions. Those animals however, which were allowed to survive for a sufficiently long period on Diet 11, developed flabbiness, muscular weakness, and a break in the growth curve, followed by rapid decline and death. At autopsy they showed very characteristic gross and microscopic lesions in the muscles.

Table V shows that five guinea pigs, killed after 31 to 75 days on the diet, had normal muscles, while six, that were allowed to survive as long as possible, showed well defined and extensive muscular dystrophy.

In rabbits the onset and severity of the muscular disorder was found to be the same for animals on the untreated Diet 11 as on Diet 13. Table V includes five rabbits, four of which had typical symptoms, gross lesions, and extensive alterations in the muscle. The fifth one, Rabbit 7, was killed after 30 days on the diet, before it had exhibited any symptoms.

The similarity in the growth curves of rabbits on Diet 11 and on Diet 13 with and without wheat germ oil, shown in Chart 5, illustrates the ineffectiveness of vitamin E administration in controlling the muscular disorder.

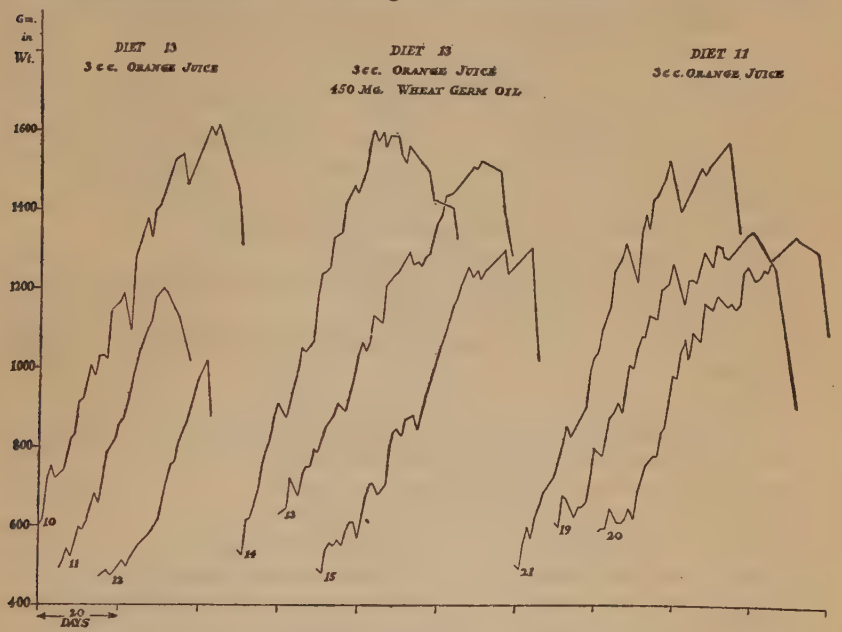



CHART 5. Growth curves of rabbits on Diet 13 and 3 cc. of orange juice, with and without a daily supplement of 450 mg. of wheat germ oil; and of rabbits on untreated Diet 11 and 3 cc. of orange juice.

It seems evident that the addition of vitamin E to Diet 13 failed to protect guinea pigs and rabbits against muscular dystrophy, and that the ethereal ferric chloride treatment of the diet plays no rôle in the production of the lesions.

The possible protective effect of green foodstuffs is now being investigated.

## CONCLUSIONS

A diet is described, which leads to a progressive, highly selective, and ultimately fatal dystrophy of the voluntary muscles. Guinea

pigs and rabbits are susceptible, rats resistant. The diet used is complete in known requirements, except for vitamin E; the addition of this factor, however, does not prevent the development of the disease. The lesions are not due to inanition, infection, or scurvy, and must be referred to some still unknown factor.

It is a pleasure to acknowledge the continued interest and advice of Dr. H. T. Clarke and Dr. G. L. Foster in this investigation.

## BIBLIOGRAPHY

1. Waddell, J., and Steenbock, H., *J. Biol. Chem.*, 1928, **80**, 431.
2. Brody, S., *Missouri Agric. Exp. Station Research Bull.*, 1926, **96**, 142.
3. Stockard, C. R., and Papanicolaou, G. N., *Am. J. Anat.*, 1918, **22**, 225.
4. Forbus, W. D., *Arch. Path.*, 1926, **2**, 318, 486.
5. Kremer, J., *Virchows Arch. path. Anat.*, 1929–30, **274**, 729.
6. Höjer, A., *Acta pediat.*, 1923–24, **3**, suppl., 68.
7. Meyer, A. W., and McCormick, L. M., *Stanford Univ. Pub.*, 1928, **2**, 2.
8. Dalldorf, G., *J. Exp. Med.*, 1929, **50**, 293.
9. Sherman, H. C., LaMer, U. K., and Campbell, H. L., *J. Am. Chem. Soc.*, 1922, **44**, 165.
10. Höjer, A., *Brit. J. Exp. Path.*, 1926, **7**, 356.
11. Goettsch, M., and Key, K. M., *Quart. J. Pharm. and Allied Sc.*, 1928, **1**, 168.
12. Eddy, W. H., *Am. J. Pub. Health.*, 1930, **19**, 1309.
13. Dalldorf, G., and Zall, C., *J. Exp. Med.*, 1930, **52**, 57.
14. Mori, S., *Bull. Johns Hopkins Hosp.*, 1922, **33**, 357.
15. Wolbach, S. B., and Howe, P. R., *J. Exp. Med.*, 1925, **42**, 753.
16. Evans, H. M., and Bishop, K. S., *J. Metabol. Research.*, 1922, **1**, 335.
17. Evans, H. M., and Burr, G. O., The antisterility fat soluble vitamine E, Memoirs of the University of California, Berkeley, 1927, **8**, 25.
18. Evans, H. M., and Burr, G. O., *J. Biol. Chem.*, 1928, **76**, 273.
19. McCollum, E. V., Simmonds, N., and Becker, J. E., *J. Biol. Chem.*, 1922, **53**, 313.

## EXPLANATION OF PLATES

### PLATE 18

FIG. 1. Guinea Pig 126. 133 days on Diet 11 plus orange juice. Section of abdominal muscles, showing hyaline necrosis of fibers, with segmentation and disruptions.

FIG. 2. Rabbit 1. 14 days on Diet 13 plus orange juice. Necrotic fiber in center. Marked proliferation of muscle nuclei between still intact fibers.

FIG. 3. Rabbit 20. 56 days on Diet 11. Invasion of necrotic fiber by polymorphonuclear leucocytes.

FIG. 4. Rabbit 1. 14 days on Diet 13 plus orange juice. Regenerating fibers.

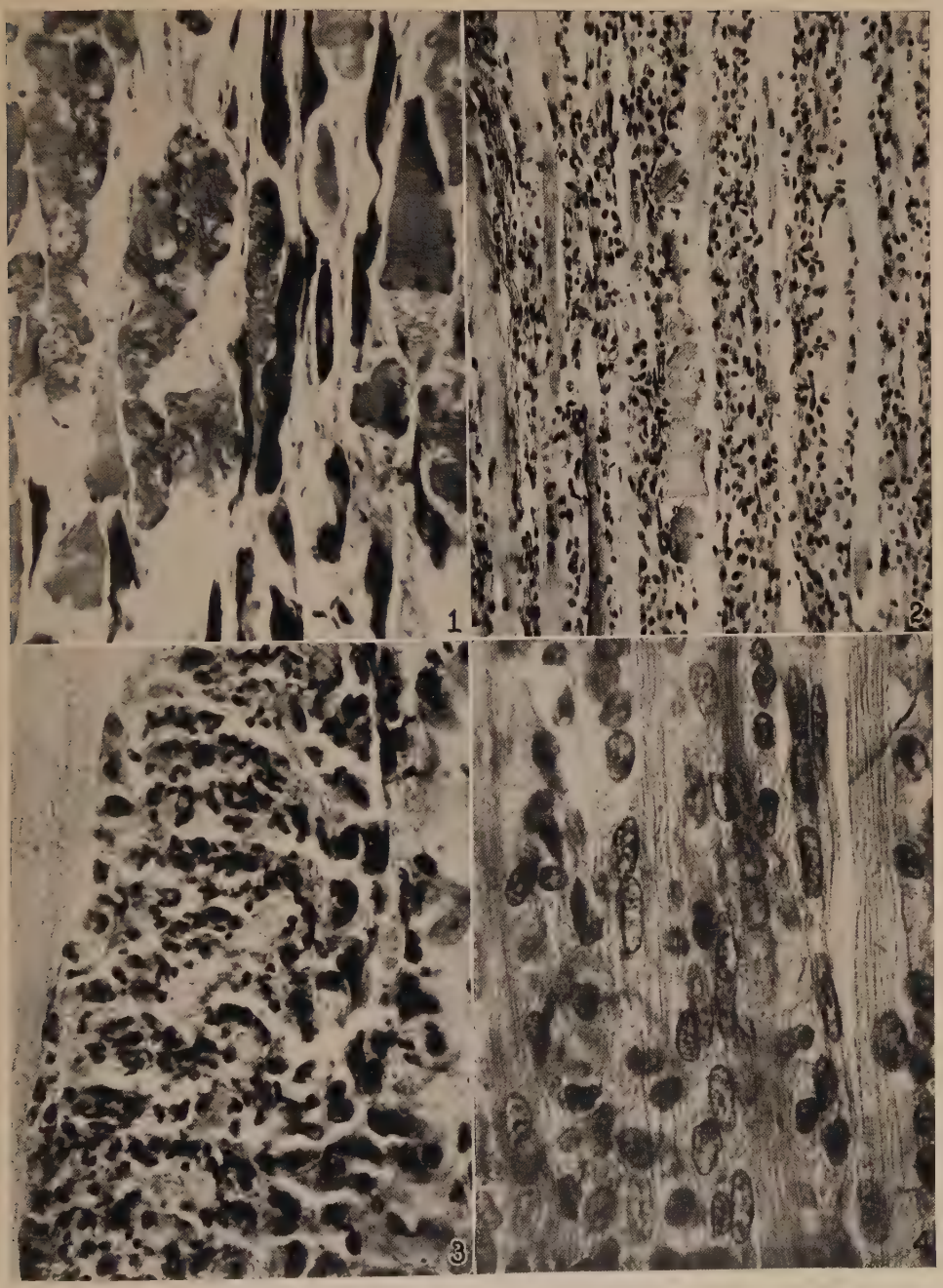
PLATE 19

FIG. 5. Guinea Pig 113. 51 days on Diet 13 plus orange juice. Extreme degeneration and loss of muscle fibers, multiplication of muscle nuclei, and formation of large multinucleate plasmatic masses.

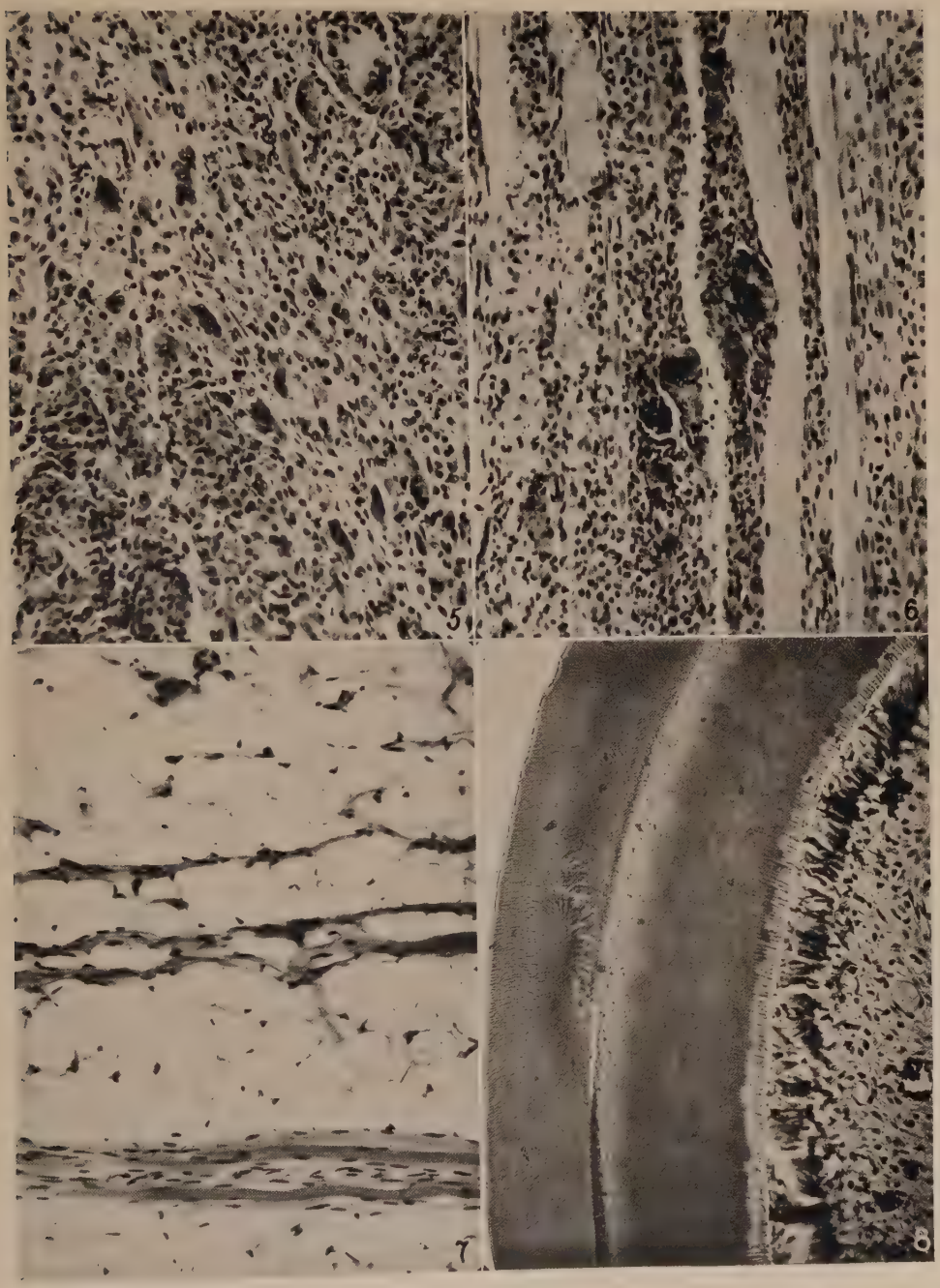
FIG. 6. Rabbit 15. 55 days on Diet 13 plus wheat germ oil. Calcification of necrotic fibers.

FIG. 7. Guinea Pig 109. 218 days on Diet 13. Quadriceps femoris muscle, showing extreme atrophy of muscle fibers and replacement with adipose tissue.

FIG. 8. Guinea Pig 58. 122 days on Diet 13 plus 3 cc. orange juice. Section through incisor root, showing normal pulp, odontoblasts, predentine, dentine, and enamel.




(Goettsch and Pappenheimer: Nutritional muscular dystrophy)




(Goettsch and Pappenheimer: Nutritional muscular dystrophy)

## BIOGRAPHICAL.

Marianne Goettsch was born in Walcott, Iowa, on March 28, 1895, and attended the public schools of Chicago, St. Louis, and Cincinnati. Chemistry and Biology were the major interests in college, and degrees were granted by the University of Cincinnati; B. A. in 1917, and M. A. in 1918.

After having held positions as analytical chemist for Sears, Roebuck and Company in Chicago, research assistant in metallography at the New Jersey Zinc Company in Palmerton, Pennsylvania, research assistant in the Department of Physiology at the University of Cincinnati, and research assistant in Nutrition in the Anatomical Department of the University of California, she was given a commission by the Pharmaceutical Society of Great Britain to carry out a problem in their Pharmacological Laboratories with Dr. Katherine H. Coward. The years 1928-1930 were spent in research and laboratory teaching in the Department of Biological Chemistry of Columbia University.

## PUBLICATIONS.

The Nutritional Requirements of Nursing Mothers. The Effect of a Deficiency of the Antirachitic Vitamin only, in the Diet of the Mothers upon the Development of Rickets in the Young.
Agnes H. Grant and Marianne Goettsch, Amer. Jr. Hygiene, 1926, 6, 211-227.

The Determination of the Antiscorbutic Value of Foodstuffs by Hojer's Method.
Marianne Goettsch, Appendix by K. M. Key, Quar. Jr. Pharm. & Allied Sciences, 1928, 1, 168-177.

The Oestrous Cycle in the Guinea Pig and the Siutability of the Uterus for the Estimation of Pituitary Extract.
Marianne Goettsch, Quar. Jr. Pharm. & Allied Sciences, 1928, 1, 34-36.

A Cerabellar Disorder in Chicks, Apparently of Nutritional Origin.
Alwin M. Pappenheimer and Marianne Goettsch, Jr. Exp. Med., 1931, 53, 11-26.